p, 4 H, app J = 4.1 Hz, CH_2NHCH_2), 3.08 (d, 2 H, J = 7.6 Hz, Phe CH_2), 3.28 (app q, 2 H, app J = 4.1 Hz, (C=O)-$NHCH_2CH_2NH$), 3.55 (t, 2 H, J = 5.4 Hz, $NHCH_2CH_2OH$), 4.42 (dd, 1 H, J = 7.6 and 17.2 Hz, α-CH), 5.01 (s, 2 H, CBZ CH_2), 5.70 (d, 1 H, J = 8.0 Hz, CBZ-NH), 6.54 (t, 1 H, J = 4.1 Hz, Phe-NH), 7.24 (m, 10 H, ArH).

Conversion of **3b** to the corresponding alkyl chloride with $SOCl_2$, reaction with EDA, acylation by 3.3 equiv of 4-phthalimidobutyryl chloride,[41] and removal of the CBZ group gave 1-(phenylalanyl)-4,7,10-tris(4-phthalamidobutyryl)-1,4,7,10-tetraazadecane (**3c**): ^{1}H NMR (D_2O/HOAc-d_4) δ 1.85 (m, 6 H, $CH_2CH_2CH_2$), 2.40 (m, 6 H, $NCH_2CH_2CH_2C$=O), 3.36 (m, 20 H), 4.25 (t, 1 H, J = 8.0 Hz, α-CH), 7.29 (m, 5 H, Phe ArH), 7.74 (m, 12 H, phthalyl ArH).

Coupling of **3c** to CBZ-Tyr-Gly-Gly-Gly followed by deprotection of phthalyl and CBZ groups, conversion to the HCl salt, and recrystallization from anhydrous 2-propanol/methanol, gave 1.0 g (6% overall yield) of **3a** as a white solid: mp 230-234°C dec; $[\alpha]_D^{25}$ = 20.7° (c = 3.3, MeOH); ^{1}H NMR (D_2O, CH_2Cl_2 int std) δ 1.79 (br m, 6 H, $CH_2CH_2CH_2$), 2.24 (app q, 2 H, J = 7.3 Hz, $NCH_2CH_2CH_2(C$=O)NH), 2.38 (br m, 4 H, $NCH_2CH_2CH_2(C$=O)N), 2.88-3.30 (br m, 22 H), 3.74 and 3.78 (2 s, 2 H each, Gly CH_2), 4.14 (t, 1 H, J = 6.8 Hz, Tyr α-CH), 4.40 (br t, 1 H, J = 6.5 Hz, Phe α-CH), 6.74 and 7.04 (2 d, 2 H each, J = 6.8 Hz, Tyr ArH), 7.20 (m, 5 H, Phe ArH). An analytical sample was prepared by reverse-phase HPLC (k' = 2.83). Anal. ($C_{40}H_{63}N_{11}O_8 \cdot 4HCl \cdot 5H_2O$) C, H, N.

1,4,7-Tris(4-aminobutyryl)-1,4,7-triazaheptane Trihydrochloride (5). To 1.5 g (6.0 mmol, 3.5 equiv) of 4-phthalimidobutyryl chloride[41] in 40 mL of anhydrous THF containing 2.4 mL (60.0 mmol) of Et_3N cooled to -78 °C was added dropwise over a 30 min period 0.18 mL (1.7 mmol) of diethylenetriamine in 5 mL of anhydrous THF under a N_2 atmosphere. After addition was complete the reaction was allowed to warm to room temperature, then filtered, and the filtrate was concentrated in vacuo. Recrystallization from $CHCl_3/Et_2O$ yielded 800 mg (63%) of 1,4,7-tris(4-phthalimidobutyryl)-1,4,7-triazaheptane: ^{1}H NMR ($CDCl_3$) δ 1.99 (br p, 6 H, J = 5.9 Hz, $CH_2CH_2CH_2$), 2.23 (dt, 2 H, J = 5.9 Hz, $NCH_2CH_2CH_2(C$=O)NH), 2.42 (t, 2 H, J = 5.9 Hz, $NCH_2CH_2CH_2(C$=O)N), 3.40 (br s, 8 H), 3.70 (m, 6 H, $NCH_2CH_2CH_2(C$=O)), 6.72 and 6.84 (2 br s, 1 H each, amide NH), 7.73 (m, 12 H, phthalyl ArH).

Removal of the phthalyl groups was as for **1d**. The final product was crystallized as the 3HCl salt from EtOH/i-PrOH, giving ~200 mg (25% overall yield) of **5** as a very hygroscopic material (no combustion analysis could be obtained): ^{1}H NMR (D_2O, $CHCl_3$ int std) δ 1.48 (p, 6 H, J = 7.6 Hz, $CH_2CH_2CH_2$), 1.90 (app q, 4 H, app J = 7.3 Hz, $CH_2CH_2CH_2(C$=O)NH), 2.12 (t, 2 H, J = 7.3 Hz, $CH_2CH_2CH_2(C$=O)N), 2.55 (app q, 6 H, J = 7.8 Hz, $CH_2NH_3^+$), 3.00 (m, 8 H).

Bioassays. The affinity of the synthetic compounds for opioid receptor types in brain membranes from male Hartley guinea pigs was assessed by standard radioligand competitive binding at 25 °C in the presence of 100 mM NaCl, as previously described.[44] The final concentrations of labeled ligands used were as follows: 0.5 nM [^{3}H]naloxone (μ-binding), 0.7 nM [^{3}H]DADLE in the presence of 4 nM sufentanil (δ-binding), and 1 nM (-)-[^{3}H]EKC in the presence of 500 nM DADLE and 20 nM sufentanil (κ-binding). The activity of the synthetic compounds was not changed upon addition of 50 μg/mL bacitracin (data not shown). The pharmacologic activity of the compounds was assessed with the electrically stimulated intact ileum from male albino Hartley guinea pigs at 37 °C as previously described.[40]

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Monoterpenic Fragment Analogues of Aplasmomycin as Potential Antimalarials

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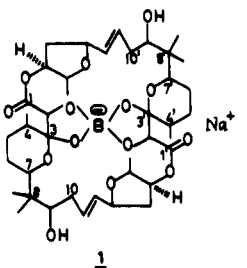
Seven analogues of monoterpenic fragment of aplasmomycin were synthesized as targeted antimalarial agents. The potency of the compound **6** was comparable with the sesquiterpene lactone artemisinin and the antibiotic aplasmomycin in vivo against *Plasmodium berghei yoelli*.

Aplasmomycin (**1**), a boron-containing ionophoric antibiotic isolated from marine *Streptomyces griseus*, was found to be active against *Plasmodium berghei* (NK 65) in vivo.[1] Several cyclic sesquiterpene peroxides[2,3] also exhibit antimalarial activity against a variety of parasite strains and the most notable are the endoperoxide sesquiterpene lactone artemisinin (**2**) and its derivatives.[4-7] The efficacy of these sesquiterpene derivatives was attributed in part on the observation that the parasitized red cells are selectively damaged by the oxidants, suggestive of an oxidative mechanism.[8,9] The presence of the terpenoidal moiety in **1** and **2** led us to the investigation of the structure-antimalarial activity relationship of monoterpenic fragment analogues of aplasmomycin.



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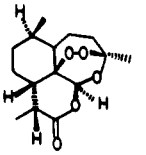

2

In the present investigation seven analogues (**4-10**) of monoterpenic aplasmomycin fragment and a hemiterpenic ester (**3**) were synthesized and evaluated for antimalarial

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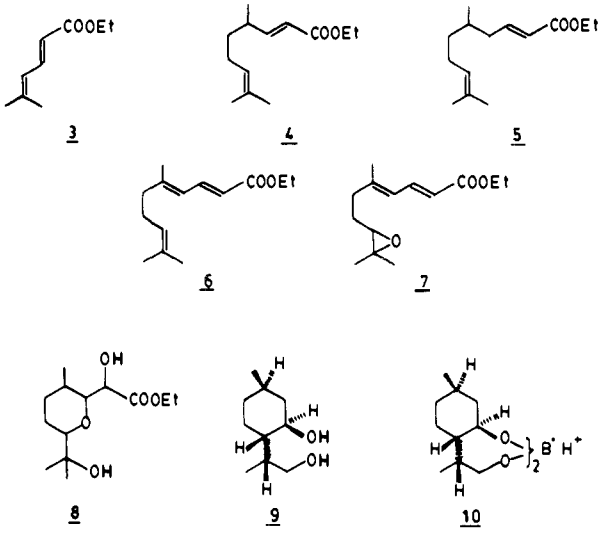
Table I. Antimalarial Activity of Compounds 2–10 against *P. berghei* in Vivo

compd.	mean survival time (days)[a] for 20, 40, 80, 160 mg/kg dose	control days	remarks[b]
2	10.8, 12.8, -, -	6.0	A***
3	5.4, 6.0, 7.4, 8.8	6.2	I
4	5.4, 6.0, 7.2, 8.0	6.2	I
5	6.0, 8.2, 10.4, 12.4	6.2	A*
6	11.4, 13.2, 13.8, 15.4	6.0	A***
7	7.8, 8.6, 9.2, 12.6	6.5	A*
8	6.4, 7.2, 8.4, 11.4	6.2	I
9	5.4, 6.2, 7.0, 8.2	6.2	I
10	9.0, 9.6, 10.8, 12.6	6.5	A*

[a] IP inoculum of 1×10^6 parasitized red cells was used for infection. All mice were observed until death. Those compounds given to groups (consisting of five mice) that survived twice the period of the untreated control group were considered to be active. None of the compounds tested was found to be toxic. Artemisinin (20, 40 mg/kg), Cycloguanil hydrochloride (25 mg/kg), and Dapsone (20 mg/kg) were used as standard drugs. [b] I, inactive; A*, active at 160 mg/kg dose level; A***, active at 40, 80, 160 mg/kg dose levels.

activity in vivo against rodent model *Plasmodium berghei yoelli*.



Results and Discussion

Chemistry. The compounds 3, 4, 5, and 6 were synthesized from 3-methyl-2-butenal, 2,6-dimethyl-5-heptenal, citronellal, and citral, respectively, by Wittig reaction with triphenyl(carbethoxymethylene)phosphorane.[10] The ester 6 was converted to epoxide 7 by oxidation with *m*-chloroperbenzoic acid. The monocyclic diols 8 and 9 were obtained by permanganate oxidation of 4 in the presence of tetrabutylammonium chloride and by hydroboration–oxidation of (-)-isopulegol,[11] respectively. The chiral borate 10 was synthesized by refluxing the diol 9 and trimethyl borate in dry methanol.[12]

Biology. The antimalarial activity of the compounds 2–10 was evaluated against *P. berghei yoelli* (NK 65), a virulent strain of rodent malaria in mice, using Rane's schizonticidal method described by Osdene et al.[13]

On comparison of activities of compounds 3–7, the ester 6 was found to have highest potency (Table I). More significantly, the lack of activity of epoxide 7 at 40 and 80 mg/kg doses and the inactivity of esters 3 and 4 clearly indicate that both the isolated double bond at position 8 and the conjugated 2,4-diene ester moiety present in compound 6 are essential for displaying pronounced antiparasitic activity. Compound 6 was found to be active at a 40 mg/kg dose, which was comparable with the activity of artemisinin (2) under the same test conditions, while the antibiotic aplasmomycin (1) exhibited antimalarial activity at the 100 mg/kg dose level against *P. berghei*.[1] Among the monocyclic diols 8 and 9 and the borate ester 10, only the chiral borate 10 was found to be active at the 160 mg/kg dose level.

Table II. Secondary Screening of Compounds 2, 5, 6, 7, and 10 by Peter's Suppressive Test[14] against *P. berghei* in Vivo

compd	dose/day,[a] mg/kg	MST (days)[b] on 60th day post infection	no. of mice survived for 15–60 days post infection/total mice in each group
2	40	30.6	2/5
5	160	29.6	2/5
6	40	20.2	1/5
7	160	30.4	2/5
10	160	28.6	2/5

[a] Ip inoculum of 1×10^7 parasitized red cells was used for infection. [b] All mice were observed for 60 days post infection. The mean survival time (MST) of the infected untreated control group was 6.4 days. The surviving mice were found to be cured in all groups.

The secondary screening of active compounds against *P. berghei yoelli* was done by Peters' test by means of parasite counts. The compounds 2, 5, 6, 7, and 10 were tested at their minimum active dose levels as found in Rane's test (Table I). All the new compounds at the dose levels mentioned had activities similar to that of artemisinin (40 mg/kg, Table II).

Further applications of the present studies are in progress.

Experimental Section

Infrared spectra were run on a Perkin-Elmer Model 681 spectrophotometer. ^{1}H NMR spectra were recorded on Hitachi R-600 (60 MHz) spectrometer using deuteriated chloroform with TMS as an internal standard. Mass spectra were obtained on a Shimadzu QP-1000 mass spectrometer. Microanalyses were performed on Carlo Erba Model 1106 elemental analyzer. All melting points are uncorrected. Citral, citronellal, (-)-isopulegol, and 2,6-dimethyl-5-heptenal were obtained from S. H. Kelkar & Bros., Bombay, and Aldrich Chemical Co., respectively. Usual workup involves extraction with ether, drying over anhydrous Na_2SO_4, removal of solvent in vacuo, and chromatography of the crude product on a silica gel (100–200 mesh) column with ethyl acetate–petroleum ether (60–80 °C) as eluent to yield pure products.

General Procedure for Synthesis of Esters 3–6. To a solution of aldehyde (10 mmol) in dry toluene (25 mL) was added triphenyl(carbethoxymethylene)phosphorane (10.2 mmol), and the solution was refluxed for 16 h. Usual workup gave the conjugated ester.

Ethyl 5-methylhexa-2,4-dienoate (3): yield, 93%; IR (neat) 1710, 1630, 1380 cm^{-1}; ^{1}H NMR δ 1.28 (t, 3 H, J = 7 Hz), 1.87 (s, 6 H), 4.18 (q, 2 H, J = 7 Hz), 5.53 (d, 1 H, J = 9 Hz), 5.96 (d, 1 H, J = 12 Hz), 7.20 (m, 1 H); MS m/e 154 (M^+). Anal. ($C_9H_{14}O_2$) C, H.

Ethyl 4,8-dimethylnona-2,7-dienoate (4): yield, 95%; IR (neat) 1725, 1640, 1380, 1370 cm^{-1}; ^{1}H NMR δ 1.06 (d, 3 H, J = 7 Hz), 1.30 (t, 3 H, J = 7 Hz), 1.60 (s, 3 H), 1.70 (s, 3 H), 1.80–2.60 (m, 5 H), 4.20 (q, 2 H, J = 7 Hz), 5.10 (bt, 1 H, $W/2$ = 12 Hz), 5.75 (d, 1 H, J = 16 Hz), 6.90 (dd, 1 H, J = 8 and 16 Hz); MS m/e 210 (M^+). Anal. ($C_{13}H_{22}O_2$) C, H.

Ethyl 5,9-dimethyldeca-2,8-dienoate (5): yield, 90%; IR (neat) 1720, 1650, 1370 cm^{-1}; ^{1}H NMR δ 0.95 (d, 3 H, J = 6 Hz), 1.30 (t, 3 H, J = 7 Hz), 1.62 (s, 3 H), 1.69 (s, 3 H), 1.85–2.5 (m,

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7 H), 4.20 (q, 2 H, J = 7 Hz), 5.12 (bt, 1 H, $W/2$ = 12 Hz), 5.82 (d, 1 H, J = 15 Hz), 6.95 (m, 1 H), MS m/e 224 (M^+). Anal. ($C_{14}H_{24}O_2$) C, H.

Ethyl 5,9-dimethyldeca-2,4,8-trienoate (6): yield, 90%; IR (neat) 2980, 1730, 1640, 1370 cm^{-1}; ^1H NMR δ 1.24 (t, 3 H, J = 7 Hz), 1.60 (s, 3 H), 1.66 (s, 3 H), 1.86 (s, 3 H), 1.98–2.40 (m, 4 H), 4.18 (q, 2 H, J = 7 Hz), 5.10 (bt, 1 H, $W/2$ = 12 Hz), 5.75 (d, 1 H, J = 15 Hz), 5.98 (d, 1 H, 12 Hz), 7.60 (dd, 1 H, J = 12 and 15 Hz); MS m/e 222 (M^+). Anal. ($C_{14}H_{22}O_2$) C, H.

Ethyl 8,9-Epoxy-5,9-dimethyldeca-2,4-dienoate (7). The epoxide **7** was synthesized by treatment of **6** (1 mmol) with *m*-chloroperbenzoic acid (1.4 mmol) in chloroform at 0–5 °C for 3 h, followed by usual workup: yield, 95%; IR (neat) 1715, 1640, 1380 cm^{-1}; ^1H NMR δ 1.26 (t, 3 H, J = 7 Hz), 1.28 (s, 6 H), 1.90 (s, 3 H), 1.50–2.50 (m, 4 H), 2.75 (t, 1 H, J = 7.5 Hz), 4.22 (q, 2 H, J = 7 Hz), 5.82 (d, 1 H, J = 15 Hz), 6.08 (d, 1 H, J = 12 Hz), 7.58 (dd, 1 H, J = 12 and 15 Hz); MS m/e 238 (M^+). Anal. ($C_{14}H_{22}O_3$) C, H.

Ethyl 2-Hydroxy-2-[tetrahydro-6-(1-hydroxy-1-methylethyl)-3-methylpyran-2-yl]ethanoate (8). A mixture of compound 4 (14.2 mmol), $KMnO_4$ (25.3 mmol), and tetrabutylammonium chloride (75 mg) in acetone (300 mL) and water (75 mL) was stirred for 1 h at 0–10 °C. Acetone was removed in vacuo and usual workup gave product **8** in 50% yield: mp 118–120 °C (recrystallized from ethyl acetate–petroleum ether 1:1); IR ($CHCl_3$) 3520, 3040, 1740, 1380 cm^{-1}; ^1H NMR ($CDCl_3$ + D_2O) δ 0.96 (d, 3 H, J = 6 Hz), 1.15 (s, 3 H), 1.25 (s, 3 H), 1.29 (t, 3 H, J = 7 Hz), 1.50–2.50 (m, 5 H), 3.70–4.10 (m, 3 H), 4.25 (q, 2 H, J = 7 Hz); MS m/e 260 (M^+). Anal. ($C_{13}H_{24}O_5$) C, H.

(−)-(1*R*,3*R*,4*S*,8*R*)-*p*-Menthane-3,9-diol (9). The diol **9** was obtained by the hydroboration oxidation of (−)-isopulegol as described by Schulte-Elte et al.[11]

Bis[(−)-(1*R*,3*R*,4*S*,8*R*)-*p*-Methane-3,9-diol] Borate (10). The borate was synthesized by refluxing the diol **9** (2 mmol) in dry methanol with trimethyl borate (10 equiv) for 5 h: yield, 98%; mp 96–98 °C (recrystallized from ethanol/petroleum ether 1:4), $[\alpha]^{24}_D$ = −17.90° (c = 5.25, MeOH); IR ($CHCl_3$) 1460, 1380 cm^{-1}; ^1H NMR δ 0.93 (d, 3 H, J = 6 Hz), 0.98 (d, 3 H, J = 6 Hz), 1.15–2.20 (m, 9 H), 3.10–3.50 (m, 1 H), 3.63 (d, 2 H, J = 4 Hz); MS m/e 351 (ion). Anal. ($C_{20}H_{36}O_4B^-$) C, H.

Biological Methods. Rane's Test. Compounds **2–10** were evaluated for their activity against a virulent strain of *P. berghei yoelli* (NK 65), using Rane's schizonticidal method described by Osdene et al.[13]

Four-week-old male mice weighing 20–25 g each received intraperitoneal inoculum of 1 × 10^6 parasitized *P. berghei* red cells. The test solutions of synthesized compounds in distilled water were prepared by homogenization with two drops of 1% Tween-80 and injected once subcutaneously 72 h post infection. A control group of infected mice that were not administered any drug was kept as untreated control. The dose range selected was 20, 40, 80, and 160 mg/kg and a minimum of five mice per dose were used. Artemisinin (20, 40 mg/kg), Cycloguanil hydrochloride (25 mg/kg), and Dapsone (20 mg/kg) were kept as standard drugs in trials for comparison. Death occurring within 24 h of treatment classified as death due to toxicity. All mice receiving the drug showing survival times of 12–18 days were followed up for the presence of parasite smears. Testing was evaluated by calculating mean survival time (MST) of the treated and control groups of mice.

Peters' Suppressive Test. After the preliminary screening by Rane's test, the active compounds **5**, **6**, **7**, **10**, and artemisinin (**2**) were subjected to Peters' 4-day suppressive test[14] at their minimum active doses.

Each mouse received an intraperitoneal inoculum of 1 × 10^7 parasitized red blood cells on day D + 0 and was treated once daily from day D + 0 to D + 3. A suspension of test compound was prepared in 1% Tween-80 distilled water and administered orally after 4 h of infection. A control group of infected mice that were not administered any drug was kept as untreated control. The doses selected were 40 mg/kg for compounds **2** and **6** and 160 mg/kg for compounds **5**, **7**, and **10**, respectively. A minimum of five mice were used in each experiment. The blood films were prepared from tail-blood and stained. The blood smears were prepared from day D + 4 and then on 6, 8, 10, 13, 16, 25, 30, 40, and 60 days.

The mean survival time (MST) of the untreated control group was found to be 6.4 days.

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Registry No. **3**, 10231-96-6; (±)-**4**, 135074-35-0; (±)-**5**, 135074-36-1; **6**, 18049-09-7; (±)-**7**, 135074-37-2; **8**, 135074-38-3; **9**, 13834-07-6; **10**, 135074-39-4; (−)-isopulegol, 89-79-2; 3-methyl-2-butenal, 107-86-8; 2,6-dimethyl-5-heptenal, 106-72-9; citronellal, 106-23-0; citral, 5392-40-5.